THE UNIVERSITY OF ALBERTA

INFLUENCE OF TOWER RAPESEED MEAL AND OTHER DIETARY FACTORS ON PERFORMANCE AND LIVER COMPOSITION OF LAYING CHICKENS

by

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A THESIS

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ABSTRACT

Rations containing different levels of Tower rapeseed meal (RSM), with or without added lipotropic factors, were fed to Shaver Starcross 288 laying hens to study the effects on productive performance, liver composition and the incidence of haemorrhagic liver syndrome (HLS). A study was also conducted to determine the effect that feeding low-carbohydrate diets might have on liver composition and fat reserves of hens at the end of their first laying year. A further experiment was conducted to determine whether the effects noted with low-carbohydrate rations were related to restricted caloric intake. The composition of the livers was also determined.

The inclusion of 5, 10 or 15 percent Tower RSM in a laying ration had no detrimental effects on rate of egg production nor was there a significant effect on level of mortality or incidence of HLS in laying hens. Birds fed Tower RSM had significantly lower egg weights than those of hens fed the control rations but the differences were small. Supplementation with lipotropic factors resulted in significantly higher Haugh unit values than were obtained in the unsupplemented groups. Neither the addition of Tower RSM nor the use of lipotropic factors had any effect on the visual fat score or on moisture, fat or protein content of the livers. Assessing the fat content of the livers by using a visual score was found to account for a significant amount of the variation of the actual liver fat content.

The use of low-carbohydrate rations resulted in reduction in rate of egg production. Since only the group fed an egg ration consumed enough to meet their energy needs, the decrease in production in the other groups appeared to be related to the level of feed restriction that occurred. When the egg ration was fed there was no change in body weight or liver composition but when the other low carbohydrate rations were fed there was a decrease in body weight and liver fat content and an increase in liver protein. This suggests that a low-carbohydrate diet *per se* does not cause weight loss unless accompanied by restriction of caloric intake.

Feed restriction imposed by limiting daily feed allowances failed to show as severe effects as were noted in the previous experiment in which low-carbohydrate rations were fed. No effects on body weight or production rate were observed. As feed restriction was increased a trend toward lower liver fat levels was observed.

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INTRODUCTION

A disorder known as haemorrhagic liver syndrome (HLS) has caused heavy losses in many flocks of laying chickens. As the name implies, the disorder is characterized by the occurrence of haemorrhages in the livers of affected birds. Since the disorder may appear sporadically and causes heavy losses in severe outbreaks, information that may lead to better control and understanding of the condition would be of great interest to the poultry industry.

HLS is a very complex disorder. When the condition, or one very similar to it, was first described, it was called fatty liver syndrome (FLS). It was later called fatty liver haemorrhagic syndrome and haemorrhagic fatty liver. The different names that have been used suggest that there may be some variability in symptoms of the disorder. When the livers of affected birds are examined they are usually pale yellow in color and friable and appear to contain a high level of fat. However, haemorrhages may also occur in livers of low fat content.

Mortality from HLS is caused by a massive haemorrhage which results in rupture of the Glisson's capsule of the liver and allows the blood to pour out into the body cavity. When the livers of other birds in affected flocks are examined, there is evidence that non-fatal haemorrhages have occurred. Haematomas may be seen on one or both lobes of the liver and may vary greatly in size. Some are only pin-point in size while others may be very large.

Several factors that may influence the occurrence of HLS have been suggested. These have included excessive energy consumption, deficiencies of lipotropic factors, strain of chicken, and the environment in which the birds are kept. Recently, it has been shown that inclusion of high levels of a high-glucosinolate rapeseed meal in laying rations may increase the incidence of the disorder but the level of fat in the livers was not affected by the level of rapeseed meal fed. No studies on the influence of meals derived from the new low-glucosinolate varieties of rapeseed have been reported.

Since a number of factors may be involved in the occurrence of HLS and since the use of rapeseed meal in laying rations has been implicated in the appearance of the syndrome, it seemed desirable to study the disorder in greater detail. Consequently, experiments were undertaken to study the effects of various levels of Tower rapeseed meal and the addition of lipotropic factors on the occurrence of HLS, and the levels of fat in the livers of laying hens. Other trials were conducted to assess the influence of feeding low-carbohydrate diets or restricting feed intake on the composition of livers of laying hens.

LITERATURE REVIEW

A. Haemorrhagic liver syndrome

i. Symptoms

Haemorrhagic liver syndrome in laying hens, or a condition very similar to it, was first reported by Couch (1956) who called it "fatty liver syndrome". The disorder was characterized by increased mortality caused by massive haemorrhages in livers containing high levels of fat.

The external symptoms seen in flocks in which FLS occurred tended to be general in nature. Flocks suffering from the condition often had body weights greater than would normally be anticipated for their age and stage of production , sometimes being as much as 25-30 percent heavier (Couch, 1956; Reedy, 1968, cited by Couch, 1968). Reedy (cited by Couch, 1968) found that the combs of affected hens sometimes exhibited a scaliness or light dandruff and were pale and cool to the touch. Although healthy in appearance, birds in affected flocks usually showed a decrease in rate of egg production. As long as the hen continued to lay at a high rate, mortality from FLS was low (Couch, 1956). In affected flocks, minor disturbances such as weighing the birds sometimes were sufficient to trigger death. Incidence of the disorder tended to be higher during the warmer months.

The internal symptoms seen on post-mortem examinations included fatty livers and excessive deposits of abdominal fat, with both the intestinal mesentery and kidneys heavily coated with fat. The livers were usually greatly enlarged,

light yellowish-brown in color and had a friable consistency. Haemorrhages and haematomas, both recent and old, were usually present. Some were large and others small and were found on either one or both lobes of the liver. Some of the larger haematomas contributed to the unnaturally large size of the liver (Couch, 1956; Wolford et al., 1971). Microscopic sections of affected livers revealed fat infiltration of the parenchyma cells of the liver (Ringer and Sheppard, 1963; Reedy, 1968; cited by Couch, 1968). Blum et al. (1973) attributed the cause of death to a massive haemorrhage following the rupture of the Glisson's capsule of the liver. It was suggested that the friable nature of affected livers made them very fragile and susceptible to injury from the least stress or pressure.

Birds with FLS usually showed high levels of fat in the liver. Lipid levels ranging from 46 to 83 percent of the liver on a dry matter basis have been reported (Couch, 1956; Nesheim et al., 1969). Tudor (1967) suggested that mortality was due largely to circulatory stress catalyzed by excessive fat accumulation. A study carried out by Wolford and Murphy (1972) showed that livers having less than 4 grams of lipid per liver or weighing less than 30 grams wet weight did not develop haemorrhages. This did not imply that lipid level and liver size as such were the causes of haemorrhaging since not all of the hens with liver lipid values and weights greater than those specified developed haemorrhages.

The occurrence of FLS does not follow any consistent pattern. In some instances FLS has occurred more frequently in caged layers than in hens originating from the same population kept in floor pens; in other instances, flocks originating from the same hatch and using identical feeding and management techniques showed varying levels of the disorder (Reedy, 1968; cited by Couch, 1968). In general, it has been observed that heavier hens tended to be more prone to develop the disorder (Barton et al., 1966; Blum et al., 1973).

The interrelationships between liver fat content and incidence of FLS are not well defined. Thayer et al. (1973), monitored the composition of liver lipids from laying hens and concluded that an elevated level of triglycerides (TG) was responsible for the increase in total lipid concentration of the liver. During the first 4 weeks of lay there was a drastic decrease in the palmitate and stearate fatty acid concentrations accompanied by an equally dramatic rise in oleate and linoleate fatty acid levels in the liver. The phospholipid content had no significant effect on the increase in total lipid concentration. Percentages of cholesterol and cholesteryl ester in the dry liver shifted very little relative to age or lipid concentration. The total lipid concentration in the liver appeared to reach a peak when the hens were 48 weeks old. There was a distinct difference in the percentage increase of TG concentration in the liver between hens with livers having a total lipid concentration

above or below 25 percent. In hens with a total lipid concentration that exceeded 25 percent in the liver, the percentage TG concentration showed a much greater increase than those with lower lipid concentration. It was suggested that alterations in fatty acid biosynthesis were the major sources of increased lipid levels in fatty livers. No correlation was found between egg production rate and liver fat level; thus, high levels of liver fat were not indicative of reduced production in a flock. Hens from apparently healthy flocks showed a very wide range of liver fat content but there was no evidence that hens possessing high levels of liver fat were unhealthy. Nevertheless, it appeared that some of the hens with high levels of liver fat were more susceptible to death from liver haemorrhage.

There is a natural increase in the formation of fat in the liver during the laying period so that it is difficult to recognize the stage at which the fat level in body tissues is extreme and causing complications. For this reason it has been suggested that the name FLS may be inaccurate. Nesheim and Ivy (1970) suggested that a more descriptive term for the condition might be "liver haemorrhage syndrome". The term "fatty liver-haemorrhagic syndrome" was suggested by Wolford and Polin (1972) as being a more inclusive term to describe the condition. Blum et al. (1973) first used the term "haemorrhagic liver syndrome" (HLS) and this term was used by Olomu et al. (1975) and Serrano (1976) as being the most appropriate for the condition involved.

ii. Factors affecting the incidence of HLS

The occurrence of HLS may be affected by numerous factors. These include nutrition of the flock, environmental conditions and genetic variations.

Many nutritional factors have been implicated in the occurrence of HLS. Of these, the effects of rapeseed meal (RSM) on the incidence of the disorder have recently received considerable attention. Hall (1972) reported that inclusion of RSM in the diet could result in the development of HLS and suggested that the condition was caused by toxic factors in the meal. It was postulated that the factors caused selective ante-mortem lysis of the reticular substance which eventually lessened the structural strength of the hepatic tissue without interfering with its vital functions. It was also suggested that even a transitory rise in blood pressure such as occurs when the hen prepares to lay an egg could result in a fatal haemorrhage. Sell et al. (1968) could not find a specific cause for increased mortality from feeding dietary RSM but noted that the rate of mortality was markedly increased if 10 percent or more RSM was included in the ration fed. Although feed consumption and feed conversion efficiency (FCE) were not significantly affected, there was a marked depression in egg size. Cardin et al. (1968) found no significant differences in hen-day egg production but observed that mortality rose steadily when levels of RSM were raised above four percent of the diet, reaching 52 percent mortality when a level of

16 percent RSM was used. Hypertrophy of the thyroid also became more pronounced at the higher level of RSM inclusion.

The use of RSM in layer rations has been restricted because inclusion of high levels of meal in the rations fed has resulted in increased mortality and decreased productive performance. As a consequence, the maximum level of RSM recommended for laying rations has been five percent (Clandinin et al., 1976).

The adverse effects of RSM on egg production may be related to the glucosinolate content of the meal or the derivatives of same. When the seed cells are crushed in the presence of adequate moisture the enzyme myrosinase hydrolyzes the glucosinolates releasing isothiocyanates, thiocyanates and nitriles in varying proportions (McGregor and Downey, 1975). Smith and Campbell (1976) speculated that the nitrile hydrolytic products of progoitrin, the major glucosinolate of the Napus varieties of RSM, influenced the formation of the liver connective tissue matrix. The severe reticulolysis induced the occurrence of liver haemorrhage. Nitrile compounds were present in all areas of the digestive tract of the hens after ingestion of RSM and egg production was drastically depressed when 50 percent of a high-glucosinolate RSM was included in the ration. The depression in egg production was significantly less when an equal level of low-glucosinolate RSM was fed. March et al. (1975), in studies on the use of RSM observed that most mortality from liver haemorrhage occurred in birds which had

been fed rations containing RSM during the growing period and the laying period as well.

In 1974 a new rapeseed cultivar called Tower was licenced for commercial production in Canada. This variety of rapeseed was developed at the University of Manitoba and featured a low-glucosinolate content as well as a low level of erucic acid in the oil. The new variety contained approximately 10 percent as much glucosinolate as varieties grown in Canada to that time (Stefansson and Kondra, 1975). Initial experiments on the use of the meal derived from the new variety suggested that a level of at least 10 percent of the meal may be incorporated in chicken laying rations without adverse effects on mortality, egg production or feed efficiency (Clandinin et al., 1976).

FLS was first noticed shortly after the energy-protein ratio had been increased in layer rations (Couch, 1956); however, changes in the energy or protein levels of the diet appeared to have little or no effect on liver fat content. Increasing the energy content of the diet while holding the protein content constant caused an increase in cholesterol levels but when the energy was held constant and the protein was increased by one percent the serum cholesterol level was reduced. Apparently, fairly wide calorie-protein ratios in the diet were tolerated by laying hens with no detrimental effects on their egg production (Miller et al., 1957; Price et al., 1957). Although the ability of individual birds to adjust to

changes in calorie-protein ratio varied, there did not appear to be any correlation between the ratio and liver fat content (Hochreich et al., 1957; Quisenberry et al., 1967; Griffith et al., 1969; Ivy and Nesheim, 1973).

The apparent derangement of fat metabolism in hens suffering from HLS indicated that there might be a deficiency of specific nutrients in the diet. Welch and Couch (1955) suggested that methionine or methylating agents might be in short supply in the high-energy laying rations, thus affecting the efficiency of energy metabolism. It was then suggested (Couch, 1956) that compounds involved in the transmethylation reactions in metabolism might influence the accumulation of fat in the livers of laying hens. On this basis, a premix designed to help reduce the liver lipid content consisting of choline, vitamin B_{12} and vitamin E was devised for addition to laying rations. This combination of vitamins was referred to as an FLS premix or a lipotropic premix. In initial studies the premix seemed to be beneficial but later trials failed to show favourable responses (Wolford and Murphy, 1972; Jensen et al., 1974).

Because of the importance of choline in transmethylation, several studies to assess the effects of choline supplementation on production, mortality and levels of fat in the livers of laying hens were conducted. Nesheim et al. (1967) studied the effect of dietary choline during the growing period on requirements during the laying period.

It was observed that choline supplementation of purified diets during the latter part of the growing period gave rise to lower egg production and increased incidence of fatty livers if the birds were placed on a choline-free diet during the laying period. Thus, it appeared that the response to choline supplementation during the laying period was affected by the choline nutrition during the growing period of the pullets. Previously, Lucas et al. (1946) and Ringrose and Davis (1946) had found that hens fed an almost choline-free diet exhibited only marginal reductions in egg production and hatchability compared to hens fed a diet containing added choline. More recent studies have also indicated that choline supplementation of laying rations was not beneficial (Nesheim et al., 1971; Chah et al., 1975). Norvell and Nesheim (1969) concluded that hens possessed a metabolic mechanism for choline synthesis sufficient to meet their requirements. Continuous feeding of between 940 and 1800 milligrams of supplemental choline per kilogram of practical corn-soybean diets gave no significant differences in egg production, hatchability, mortality or liver fat content (Bossard and Combs, 1970). Other workers (Griffith et al., 1969), however, found that liver fat levels were reduced when laying rations were supplemented with choline and their findings were supported by Couch and Grossie (1970) and Schexnailder and Griffith (1973).

In addition to choline, vitamin E and vitamin B_{12}, inositol was also shown to influence liver fat deposition (Couch, 1968; Parker, 1968; cited by Couch, 1968; Bull, 1968; cited by Couch, 1968; Reed, 1968; cited by Couch, 1968). When inositol was included in laying rations at a level of 2 pounds per ton of feed (Couch, 1968) along with the FLS premix containing choline, vitamin E, vitamin B_{12} and oxytetracycline, the fat content of the livers was effectively decreased. In addition, comb pigmentation of the hens was improved and egg production returned to normal once the liver fat content was reduced. It was also observed that there was less variation in the fat content of the livers of laying hens when inositol was included (Reed, 1968; cited by Couch, 1968). Jensen et al. (1970) found that a combination of choline chloride, inositol, vitamin B_{12} and vitamin E significantly reduced liver weight, liver fat and total liver fat accumulation in hens fed a corn-soybean meal (SBM) ration but not in those fed a wheat-pea diet.

Observations on the effects of inositol on liver fat levels have not been consistent. Some studies have shown that the addition of inositol to layer diets had no significant effects on levels of liver fat (Bossard and Combs, 1970; Nesheim and Ivy, 1970), percent total serum lipids (Ragland et al., 1970; Pearce, 1972) and body and liver weights (Leveille and Bray, 1970). The discrepancies noted suggested that inositol was not universally effective in

reducing the level of liver fat in laying hens.

More recently, there has been a growing interest in the possibility of using biotin as a lipotropic agent in laying rations because of its effectiveness in preventing a fatty liver disorder of broilers known as fatty liver and kidney syndrome (Blair and Whitehead, 1974; Payne et al., 1974; Whitehead et al., 1974a). Experiments that have been reported, however, have failed to show that biotin is effective in preventing increases in liver fat (Schexnailder and Griffith, 1973; Chah et al., 1975; Jensen et al., 1976) or the occurrence of HLS in laying hens (Serrano, 1976). The recommended level of biotin in layer rations is 0.15 mg per kilogram of diet (NAS-NRC, 1971) but administration of a 25-fold excess of biotin to laying hens had no adverse effect on liver weight or liver lipid content (Balnave, 1975).

The incidence of HLS may be influenced by the feedstuffs used in laying rations. In isocaloric diets the total liver fat was greatest in hens fed grain sorghum, corn or triticale and lowest in those fed barley, oats or rye (Jensen et al., 1976b). There were no differences in liver fat content between groups fed wheat diets and those containing corn oil or animal tallow in isocaloric diets. The dry matter and fat content of livers were greater in hens fed corn diets than in hens fed isocaloric wheat-based diets (Jensen et al., 1976b). Liver weight was found to be directly related to liver fat content (Kim et al., 1976).

Inclusion of high levels of fat in laying rations has been shown to cause an apparent derangement of lipid metabolism and increased liver fat content (Naber, 1968; cited by Couch, 1968). Changes such as elevated plasma cholesterol (Weiss and Fisher, 1957; Frank and Waible, 1960) and total plasma lipid (Weiss and Fisher, 1957), excess deposits of body fat, as well as friable and fatty livers have been reported when large amounts of animal fat or vegetable oil were included in hen diets (Weiss and Fisher, 1957; Donaldson and Gordon, 1960; Frank and Waible, 1960). Rapeseed oil added to a ration at a level of eight percent caused fatty livers but soybean oil and sunflower oil at the same level protected against accumulation of liver fat (Bragg et al., 1973).

Liver fat accumulation may be affected by the level of energy in the ration fed. Barton et al. (1966) observed that hens fed low-energy diets had lower liver fat levels than those fed a control ration of higher energy content. Only the low-energy diets (2360 kilocalories metabolizable energy per kg) prevented FLS from developing. When the hens fed the low-energy ration were placed on the control ration, liver fat levels soon increased to the same level as those fed only the control ration.

Hardness of the water used by hens may also affect the incidence of HLS (Jensen et al., 1976a). Flocks from areas with water supplies containing higher levels of minerals had a higher incidence of HLS.

Environmental factors have been implicated in the occurrence of HLS. In commercial laying flocks, liver fat values were found to be higher among caged birds than among similar birds kept in floor pens and fed the same diet (McDaniel et al., 1957; Barton et al., 1966). The observation that hens in cages gained more weight than those in floor pens suggested that a deficiency in the caged layer diet may have existed or that the restricted movement of hens in cages affected fat deposition in the birds (Price et al., 1957). Garlich et al. (1975) found highly significant differences in liver lipid levels attributable to system of housing and strain of layers. Couch (1956) observed that higher temperatures appeared to result in stress and increased liver lipid content of laying birds. A similar effect of temperature on liver lipid levels was observed by Griffith et al. (1969) and Schexnailder and Griffith (1973). It has also been reported that mortality rates were higher during the warmer months of summer (Couch, 1956; Nesheim et al., 1969; Blum et al., 1973).

Genetic variations within strains as well as between strains seem to influence lipid biosynthesis, transport, energy intake and even the degree of liver haemorrhage. The greatest differences in liver fat content occurred between strains (Nelson and Carlson, 1976). Some strains consistently had high liver lipid values and others had low liver lipid values regardless of the method of housing or feeding that was employed. There was also a wide range in

liver fat values for individual hens within each strain and feeding regimen. Nesheim and Ivy (1970) reported that of 39 hens that died from HLS, 11 were granddaughters of one sire and six were from another. Older hens also seemed more prone to HLS but it was unclear whether it was related to the stage of egg production or the natural increase in formation of fat during the laying period (May and Stadelman, 1960).

B. Methods for estimating levels of liver fat

In order to try to avoid chemical determination of liver lipid levels various methods of scoring have been attempted. Jensen et al. (1970) reported that visually scoring livers for fat accumulation was inadequate as a means of detecting treatment differences. Cunningham and Morrison (1976) observed, however, that liver color was a good estimator of liver fat content ($r = 0.81$) but liver firmness did not estimate liver fat content with much accuracy ($r = 0.39$). They stated that "neither liver color nor liver firmness was a good predictor of carcass lipid content even though almost all the de novo fatty acid synthesis takes place in the liver of the chicken." Plasma protein levels were not helpful in detecting a developing fatty liver condition (Duke et al., 1968). It was only after a fatty liver condition had developed that changes in plasma protein levels occurred.

C. Fatty liver and kidney syndrome (FLKS) in broiler chicks

FLKS is a disease of unknown aetiology which features

an increase in the amount of fat deposited in the liver, kidney and myocardium of young chickens. Peak mortality usually occurs between the 18th and 24th day of age (Hemsley, 1965; Blair et al., 1969). The disease was first described by Marthedal and Velling in 1958 (cited by Hemsley, 1965). As in HLS of laying hens, chicks suffering from FLKS had greatly increased levels of TG in the lipid content of their liver and kidneys (Evans et al., 1975). Hepatic lipogenesis was reduced but the mitochondrial functions were impaired. Livers were pale and swollen, and haemorrhages were sometimes present. Plasma-free fatty acid concentrations were invariably elevated and likely were a major factor contributing to the development of fatty deposition, especially in the extrahepatic tissues of the chick (Hemsley, 1965; Evans et al., 1975). It was observed that the majority of liver cells were packed with small fat droplets. Evans et al. (1975) suggested that the incidence of hyperlipaemia was probably owing to a reduced rate of lipid clearance rather than an increased rate of lipoprotein synthesis.

Although the exact cause of FLKS is not known many factors seem to influence the incidence of the disorder. There appears to be a relationship between the incidence of FLKS and environmental stress (Husbands and Laursen-Jones, 1969). Conditions such as weighing or disturbing the birds, increased housing temperatures or withdrawal of feed all seem to trigger an increase in mortality

(Payne, 1975; Blair and Whitehead, 1976). There also appears to be a positive correlation between the incidence of FLKS and protein content or energy-to-protein ratio of the diet (Blair et al., 1969; Whitehead and Blair, 1974). Increased mortality was observed in diets with a high energy-protein ratio . A disorder of fat metabolism has been suggested (Blair et al., 1973) since diets containing higher levels of essential fatty acids (EFA) showed a reduced level of mortality (Husbands and Laursen-Jones, 1969).

Several workers (Blair and Whitehead, 1974; Payne et al., 1974; Payne, 1975; Blair and Whitehead, 1976) have shown that supplementing chicken broiler rations with biotin was an effective means of preventing the occurrence of FLKS. It was suggested that different levels of biotin (Blair and Whitehead, 1976) or fat (Blair et al., 1973) in the grain sources for chick diets might also account for the varying incidence of FLKS. Pelleting the diets appeared to increase the incidence of FLKS suggesting that one or more nutrients present in borderline quantities such as biotin may have been destroyed or modified (Blair and Whitehead, 1974; Blair et al., 1973).

Since FLKS was not induced by feeding biotin-deficient rations it was concluded that FLKS was a biotin-responsive syndrome (Blair and Whitehead, 1974; Whitehead et al., 1974). Bannister (1976) reported that depletion of liver biotin is not the primary cause of FLKS because

there was not a greater reduction of gluconeogenic activities of the liver in chicks fed a biotin-deficient diet than in those fed a FLKS-inducing diet. He concluded that "it is clear that FLKS is quite distinct from a biotin deficiency despite the fact that both respond to the vitamin."

D. Effect of restricted carbohydrate supply on liver composition

Performance of poultry may be affected by feeding rations of low carbohydrate content or by restricting feed intake. Allred and Roehrig (1970) reported that feeding "carbohydrate-free diets" to chickens decreased glycolytic and increased gluconeogenic enzyme activity which, in turn, increased the net production of glucose by the liver.

The type of carbohydrate used may also affect its utilization by poultry (Renner, 1971). Hamilton and Mitchell (1924) showed that the chicken lacked the enzyme lactase to break down lactose to glucose and galactose and therefore lactose was not utilized. Ingestion of lactose seemed to increase water consumption and excretion in birds (Fox and Briggs, 1959). Ashcroft (1933) found that milk products decreased the pH of the contents of the large intestine of chickens. Diarrhea developed and the caecal horns were distended to 2 or 3 times their normal size. Lactose, the major milk sugar, was not the sole factor causing the diarrhea. The soluble salts in whey seemed to increase the tendency toward diarrhea (Fischer and Sutton, 1949).

The use of diets of very high protein and fat content effectively restricts carbohydrate intake. Unrestricted intake of low-carbohydrate diets have been recommended as a means for humans to lose weight (Atkins, 1972). Rajaguru et al. (1966) found that the protein content of the carcass on a dry matter basis increased and the ether extractable components decreased with an increase in dietary protein levels. Allred and Roehrig (1970) suggested that gluconeogenic processes deplete the available supply of amino acids provided by the diet.

E. Effect of feed restriction on liver composition

The possibility that incidence of HLS might be influenced by feed restriction programs has also been explored. Feed restriction significantly lowered both liver lipid percent and egg production (Couch, 1974). Withholding food altogether for a short period of time increased mortality (Payne, 1975). Enzymes involved with glucose-6-phosphate (G6P) metabolism displayed multi-behavioural activities during nutritional depletion and restoration periods (Weber et al., 1961). The enzymes do not all simultaneously change with changes in level of feed intake. Avian hepatic carbohydrate-metabolizing enzymes behave in much the same way as those of mammals under feed restriction. Blood glucose levels in the chicken are almost double those of mammals yet fasting did not influence the total body glucose mass in the hens (Belo et al., 1976). They concluded that there must be a substantial recycling of glucose

carbon in fasted chickens since glucose utilization in the chicken is rapid.

Hepatic lipogenesis is greatly depressed in fasted hens possibly because there is an insufficient level of free CoA (Yeh and Leveille, 1971). There appeared to be some protection provided against the stress of fasting if the hens were on a high-fat, high-energy diet prior to fasting (Anonymous, 1976). Restricted feeding followed by a full feeding regimen did not increase the incidence of FLHS. Wolford and Polin (1972) found the incidence of FLHS among their restricted hens to be nil when they were put back on full feed while the control group of hens fed free choice throughout the experiment had a mortality rate of 25 percent.

In contrast to the effects of feed restriction, excessive feed intake as a result of force-feeding appears to influence the occurrence of liver haemorrhages. Wolford and Polin (1974) noted that force-fed hens developed hepatic haemorrhages similar to those of FLHS. The haemorrhagic score and incidence of haemorrhages were directly related to the total daily feed intake. Carbohydrates and lipids were equally effective in inducing FLHS. Feeding various types of diets and sources of energy at levels that were 150 percent that of birds fed ad libitum induced FLHS (Polin and Wolford, 1976). They concluded that a "positive energy balance" had been induced by force-feeding hens. These findings did not correspond to those of Ivy and Nesheim

(1973) who noted that, although force-feeding caused marked increases in liver size and fat content, no mortality from liver haemorrhages occurred.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were conducted to study:

Section I : The effects of Tower rapeseed meal and lipotropic factors on productive performance and occurrence of HLS in laying hens.

Section II : The effect of low-carbohydrate diets on the composition of livers of laying hens.

Section III : The effect of restricted feed intake on the composition of livers of laying hens.

SECTION I

The effects of Tower rapeseed meal and lipotropic factors on productive performance and occurrence of HLS in laying hens.

Status of the problem

During the past few years several reports have indicated that the use of more than five percent rapeseed meal in layer rations resulted in higher levels of mortality and increased incidence of HLS. There was also an indication that the deleterious effects noted were related to the high glucosinolate content of the meals. With the advent of the new low-glucosinolate varieties, it seemed desirable to determine whether the meals derived from the new varieties could be used at higher levels than those recommended with the older high-glucosinolate meals. Consequently an experiment was conducted to determine the effect of varying levels of Tower rapeseed meal (See Review of Literature, page 9) on productive performance and incidence of HLS in laying hens. Since the occurrence of HLS has often been associated with high levels of liver fat, treatments were included to determine whether addition of a number of lipotropic factors would have any effect on incidence of HLS or on the composition of the livers of laying birds.

Experimental

Four hundred and eighty 42-week-old Shaver Starcross 288 pullets were used in this experiment. A factorial design involving four levels of Tower RSM (0, 5, 10 and 15%) fed either with or without added lipotropic factors was used to derive the data. The birds were kept in cages (2 birds per 30 x 40 cm cage), leg-banded and divided into 16 comparable groups of 30 birds each. Two groups were placed on each of the eight ration treatments.

The composition of the rations used is shown in Table 1. The rations were designed to be isocaloric and isonitrogenous. All rations contained 2695 kilocalories of metabolizable energy per kilogram of ration and 15.9 percent protein. Feed and water were supplied ad libitum. Size #3 insoluble grit was added to all rations at the one percent level. The experiment was terminated after 32 weeks on test.

Records were kept on body weight, mortality, egg production, feed consumption, egg weight, Haugh unit values and egg specific gravity. The birds were weighed at the beginning and end of the experiment. Feed consumption was recorded for each 28-day period during the experiment. Egg size was determined by weighing all eggs laid by each group on one day each week. Haugh unit values and specific gravity were determined once a month on all eggs laid on a given day.

Table 1. Percentage composition of experimental rations for laying hens

Ingredient	RATION NUMBER							
	1	2	3	4	5	6	7	8
Ground wheat (13.5% protein)	74.215	72.115	70.015	67.915	71.415	69.315	67.215	73.515
Wheat shorts	3.0	2.0	1.0	-	2.0	1.0	-	3.0
Stabilized fat	1.0	1.9	2.8	3.7	1.9	2.8	3.7	1.0
Dehydrated alfalfa	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Meat meal (55% protein)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Tower rapeseed meal (SWP)	-	5.0	10.0	15.0	5.0	10.0	15.0	-
Soybean meal (48.5% protein)	9.0	6.2	3.4	0.6	6.2	3.4	0.6	9.0
Ground limestone	7.0	7.0	7.0	7.0	7.0	7.0	7.0	7.0
Calcium phosphate (18.5% Ca; 20.5% P)	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Iodized salt	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
Manganese oxide (62% Mn)	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Zinc oxide (78% Zn)	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Vitamin premix[1]	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Lipotropic premix[2]	-	-	-	-	0.7	0.7	0.7	0.7

[1]Vitamin premix supplied the following levels per kg of ration: Vitamin A, 6000 I.U.; Vitamin D_3, 1200 ICU; Riboflavin, 3 mg; Calcium Pantothenate, 6.75 mg; Niacin, 15 mg.

[2]Lipotropic premix supplied the following levels per kg of ration: Biotin, 200 mcg; 50% Choline Chloride, 2000 mg; Vitamin B_{12}, .02 mg; Vitamin E, 20 I.U.; DL-Methionine, 1 g.

Artificial lighting was provided to give the birds 14 hours of light per day. Mortality was recorded on a daily basis. Autopsies were performed at the Provincial Veterinary Laboratory on all hens that died during the experiment.

At the conclusion of the experiment 12 birds from each treatment were randomly selected, fasted for 18 hours and sacrificed by cervical dislocation. The livers were then examined and an estimate of their fat content was made by a visual appraisal. The visual grades, ranging from 1 to 5, were the same as those used by Serrano (1976) in which scores of 1 and 2 represented livers containing 12-14 percent fat, a score of 3 pertained to livers containing approximately 25 percent fat, and livers with scores of 4 and 5 usually contained 45 percent or more of fat on a dry matter basis.

After visual scoring, the livers were removed, cleaned of adhering fat and excess blood, and weighed. They were then placed in individual plastic bags and frozen. The dry matter content of the livers was calculated by freeze-drying the livers for 72 hours to a constant weight. Freeze-dried livers were individually ground in an Oster blender and stored at -20°C until they were analyzed for protein and fat (Appendix I).

The data were subjected to analysis of variance and significance of differences were evaluated by Duncan's Multiple Range Test (Steele and Torrie, 1960) at the 0.05 level of probability. Details of the statistical analyses

are shown in Appendix II. An analysis of covariance test was also run to determine the correlation between visual fat score and the actual chemical analysis of fat (Appendix III).

Results and Discussion

A summary of the effects of different levels of Tower RSM and lipotropic factors in the rations fed on the performance of laying hens is given in Table 2.

The level of mortality was not significantly affected by level of RSM included in the rations or by the addition of lipotropic factors. HLS occurred in only one treatment and incidence was low and probably not related to the level of RSM used. This differed from an experiment conducted using a high-glucosinolate rapeseed meal (Span) in which the incidence of HLS was significantly increased when RSM was included at levels of 10 and 15 percent (Serrano, 1976). The addition of lipotropic factors appeared to have a slightly beneficial effect on total mortality but the differences observed were not significantly different.

Egg production, both on a hen-housed and hen-day basis was not affected by levels of Tower rapeseed meal (5, 10 or 15%) included in the rations as compared to the control groups. This differed from the results of Serrano (1976) in which inclusion of 10 or 15 percent high-glucosinolate RSM (Span) resulted in a significant decrease in hen-housed production.

The addition of lipotropic factors to the rations fed

Table 2. Effects of levels of Tower rapeseed meal and lipotropic factors on performance of laying hens

	Rapeseed Meal Levels									
	0%		5%		10%		15%		Average	
Lipotropic factors	-	+	-	+	-	+	-	+	-	+
Total mortality, %	5.0	3.3	8.3	3.3	10.0	5.0	8.3	5.0	7.9	4.2
Mortality, (HLS), %	0	0	0	0	3.3	0	0	0	0.8	0
Production, hen housed, %	66.4	69.2	69.5	70.3	69.0	68.8	66.0	68.3	67.8	69.2
Average, hen housed, %	67.8		69.9		68.9		67.2			
Production, hen-day, %	68.9	70.6	72.1	71.8	71.8	71.2	69.2	70.4	70.5	70.1
Feed per dozen eggs, kg	2.11	2.02	2.00	1.96	1.96	1.96	2.04	1.95	2.03	1.97
Egg weight, g	63.1^{a1}	63.4^{a}	62.8^{b}	61.6^{b}	62.6^{b}	62.2^{b}	62.4^{b}	62.5^{b}	62.8	62.8
Egg Haugh Unit Values	76.2	76.7	76.2	78.8	77.2	78.2	74.9	76.4	76.2^{a}	77.5^{b}
Average Haugh Unit Values	76.4^{ab}		77.5^{a}		77.7^{a}		75.6^{b}			
Specific gravity	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07	1.07
Initial body weight, kg	1.81	1.81	1.78	1.78	1.86	1.82	1.82	1.82	1.82	1.81
Final body weight, kg	1.96	1.90	1.89	1.88	1.91	1.95	1.83	1.89	1.90	1.90

[1]Row values with same superscript or no superscript are not significantly different by Duncan's Range Test ($P<0.05$).

in this experiment had no effect on level of egg production. Production rates in the unsupplemented and supplemented groups were very similar.

Efficiency of feed conversion was not affected by the level of RSM used in the rations or by the addition of lipotropic factors. The lack of effect on feed conversion might be expected since the rations were isocaloric and egg production rate was unaffected.

Eggs weights were significantly lower in groups fed RSM at all levels of inclusion as compared to the control group. There were, however, no differences in egg weight between the groups fed 5, 10 or 15 percent RSM.

Statistical analyses indicated that the use of 5 or 10 percent Tower RSM in laying rations resulted in improved interior egg quality, measured by Haugh unit values, as compared to those receiving 15 percent RSM. There was, however, no difference between the control group and those receiving 15 percent RSM. There is no explanation for the higher Haugh unit values noted when 5 or 10 percent RSM was added to the ration. When the data were combined, the addition of lipotropic factors resulted in a significant increase in Haugh unit values.

Specific gravity of the eggs produced was not affected by the treatments used. The values obtained were remarkably constant.

There was no evidence of treatment effect on final body weight of the birds. Average weight gains during the

experimental period ranged from 10 to 150 grams.

Table 3 shows the composition of the hen livers from each of the treatments used in the experiment. The livers showed no visual differences in fat content at the various levels of RSM inclusion. The addition of lipotropic factors had a varying influence on visual scores but the differences noted were not significant. Fresh liver weights were not significantly different at any level of Tower RSM inclusion and supplementation with lipotropic factors had no consistent influence on the liver weights. This was reflected in the data expressing liver weight as grams per 100 grams of body weight in which no differences between treatments were observed. The values obtained indicated that liver weight was approximately two percent of body weight which is in close agreement with data from a previous report (Serrano, 1976).

The dry matter, protein and fat content of the livers were similar to the controls at all levels of Tower RSM inclusion and the addition of lipotropic factors had no consistent effect on these parameters. It also appeared that addition of lipotropic factors had no effect on liver composition.

The analysis of covariance showing the relationship between visual fat scores and chemical fat determinations of the livers gave an r value of 0.58 (Appendix III). A value of this magnitude indicates that visual fat scores account for a significant amount of the variation of the actual liver fat content.

Table 3. Effects of levels of Tower rapeseed meal and lipotropic factors supplied on grades of livers and composition of livers of laying hens

	Rapeseed Meal Levels							
	0%		5%		10%		15%	
Lipotropic factors	-	+	-	+	-	+	-	+
Liver								
Visual liver score[1]	3.0	2.5	3.2	2.6	2.9	3.5	2.6	3.0
Fresh weight, g	36.9	41.9	40.0	34.3	37.4	41.2	32.4	42.8
Average, g	39.4		37.2		39.3		37.6	
g/100 g/body weight, g	1.96	2.13	2.07	1.81	1.90	1.94	1.76	2.17
Dry matter, %	27.5	26.6	28.0	26.0	26.0	30.3	26.0	30.6
Average, %	27.0		27.0		28.2		28.3	
Protein, %	65.4	68.7	65.0	71.4	70.0	62.0	70.3	62.2
Fat, %	24.0	21.7	23.8	15.8	16.7	27.3	17.6	27.8

[1]Row values with same superscript or no superscript are not significantly different by Duncan's Range Test ($P<0.05$).

Summary

Shaver Starcross 288 pullets, 42 weeks of age, were fed rations containing four levels (0, 5, 10 and 15%) of a low-glucosinolate RSM (Tower variety), either with or without added lipotropic factors, for a period of 32 weeks. The results obtained indicated that:

1. The inclusion of 5, 10 or 15 percent Tower RSM in a laying ration had no significant effect on level of mortality or incidence of HLS in laying hens. Addition of lipotropic factors appeared to reduce mortality slightly but the differences were not significant.
2. No detrimental effects on rate of egg production, calculated either on a hen-housed or a hen-day basis, were observed when Tower RSM was included in the rations at levels up to 15 percent.
3. Interior egg quality from hens fed Tower RSM was similar to that observed in the controls. The addition of lipotropic factors resulted in significantly higher Haugh unit values than was observed in the unsupplemented groups.
4. Hens fed 5, 10 and 15 percent Tower RSM had significantly lower egg weights than those of hens fed the control ration.
5. Egg specific gravity, feed per dozen eggs and final body weight were unaffected by inclusion of RSM or lipotropic factors in the rations fed.
6. Inclusion of Tower RSM in laying rations had no effect on the visual score of livers or on their composition.
7. Addition of lipotropic factors had no consistent

influence on visual liver scores or on the dry matter, protein and fat content of livers.

8. Visual appraisal of the fat content of the livers accounted for a significant amount of the variation of the actual liver fat content ($r = 0.58$).

SECTION II

The effect of low-carbohydrate diets on the composition of livers of laying hens.

Status of the problem

Since the addition of lipotropic factors had no consistent effect on visual liver score or fat content of the livers (Section I), it would be desirable that some other method be devised to control fat deposition in the liver. A recent book has advocated the use of very low carbohydrate diets as a means for humans to lose excess fat (Atkins, 1972). It, therefore, seemed possible that the use of low-carbohydrate diets might be useful in reducing fat deposition in the body and liver of laying hens. Consequently, an experiment was conducted to determine the effect that feeding low-carbohydrate diets would have on liver composition and fat reserves of hens at the end of their first laying year.

Experimental

One hundred and twenty Shaver Starcross 288 hens, 73 weeks of age and laying at a rate of about 60% were randomly selected, leg-banded, weighed and divided into six comparable groups of 20 birds each. The hens were placed in cages (2 birds per 30 x 40 cm cage) and given feed and water *ad libitum*.

The composition of the rations used is shown in Table 4. The control ration (Ration 1) was calculated to contain 2680 kilocalories of metabolizable energy per kilogram and

Table 4. Percentage composition of experimental rations for laying hens

Ingredient	Ration Number					
	1	2	3[1]	4	5	6
Ground wheat (13.5% protein)	69.925	33.925	-	-	-	-
Ground oats	5.0	-	-	-	-	-
Ground barley	5.0	-	-	-	-	-
Stabilized tallow	-	3.0	-	5.0	6.0	10.0
Dehydrated alfalfa meal	2.0	2.0	-	-	-	-
Meal meal (55% protein)	3.5	3.5	-	-	-	53.925
Herring meal	1.0	6.0	-	-	-	5.0
Soybean meal (48.5% protein)	5.0	26.0	-	-	-	-
Dried whey (12% protein)	-	-	-	-	49.425	25.0
Skim milk powder (33% protein)	-	-	-	69.425	-	-
Cooked egg (12% protein)	-	-	95.658	-	-	-
Casein (vitamin-free; 90.3% protein)	-	-	-	18.5	37.0	5.0
Promine (91.6% protein)	-	18.0	-	-	-	-
Ground limestone	7.0	6.0	3.46	6.0	6.0	--
Calcium phosphate (18.5% Ca; 20.5% P)	0.5	0.5	-	-	0.5	-
Mineral premix[2] & iodized salt	0.23	0.23	0.46	0.23	0.23	0.23
Vitamin premix[3]	0.845	0.845	0.422	0.845	0.845	0.845

[1]In this ration the mineral premix and iodized salt content is double that of the other rations and the vitamin premix content is half that of the other treatment rations.
[2]Mineral premix supplied the following levels per kg of ration: ZnO (78% Zn), 100 mg; MnO (62% Mn), 200 mg.
[3]Vitamin premix supplied the following levels per kg of ration: Vitamin A, 6000 I.U.; Vitamin D_3, 1200 ICU; 50% Choline Chloride, 200 mg; Vitamin E, 11 I.U.; Riboflavin, 3 mg; Calcium Pantothenate, 6 mg; Niacin, 15 mg; Vitamin B_{12}, 7.5 µg.

15.9 percent of crude protein. Ration 2 was similar to Ration 1 except that the protein content was increased greatly by the addition of soybean meal and promine at the expense of the grains. A major portion of the energy in the ration was still derived from carbohydrates in the grain portion of the ration. This treatment was included to assess the effect that high protein might have on liver composition and body fat reserves. A very low carbohydrate diet (Ration 3) was devised by using cooked eggs as the source of energy and protein in the ration. Eggs were prepared by breaking the eggs into aluminum trays (10 eggs per 9 x 18 x 6 cm deep container), discarding the shells, and heating the egg contents in the autoclave at 121°C for 8 minutes. At the conclusion of this cooking procedure any moisture that had collected in the trays was drained off and the eggs were then blended in a "Kitchen Aid" mixer. Minerals and vitamins were added to provide an "egg ration". This ration was stored at 5°C and was remixed every second day with freshly cooked eggs to reduce the possibility of bacterial spoilage. Each morning any of the ration remaining in the feed troughs was removed and the troughs were thoroughly washed, dried and filled with fresh feed. Because of the low energy content of the egg ration (1555 kilocalories per kilogram and 11% C.P.) it was assumed that the hens would consume approximately twice as much of the ration as the controls. In Ration 4, skim milk powder, vitamin-free casein and stabil-

ized animal fat were used to provide approximately the same level of energy and protein as in Ration 2. It was assumed that since almost all of the carbohydrate in skim milk powder is in the form of lactose and since hens lack the enzyme lactase, the carbohydrate would not be utilized. Thus the ration could be considered to be a low-carbohydrate ration for hens. Ration 5 was similar to Ration 4 except that dried whey and casein were used in place of skim milk powder. Since whey also contains lactose, it was assumed that the energy in the ration used by the bird would be derived from fat and protein. Ration 6 was similar in energy and protein content to Rations 2, 4 and 5 (2850 kilocalories per kilogram and 40% C.P.), but all of the energy and protein was supplied by meat meal, herring meal, dried whey, vitamin-free casein and stabilized tallow. Thus, the ration was very low in carbohydrate content.

Records were kept on mortality, egg production and feed consumption. The hens were weighed after 2 and 4 weeks on test. The experiment was terminated after 4 weeks on test.

At the conclusion of the experiment 10 hens from each treatment were sacrificed by cervical dislocation and their livers were visually scored for fat content and haematomas. Each liver was removed, weighed, placed in a plastic bag and stored at -20°C until analyzed for moisture, protein and fat (Appendix I). To give an indication of body fatness, the abdominal fat pads from each bird were removed and

weighed. A record was kept of the weights of the ovary and oviduct from each bird.

Duncan's Multiple Range Test was used to evaluate between-treatment differences in the experimental data. The analyses of variance were evaluated at the 0.05 level of probability (Appendix IV).

Results and Discussion

The effects of the treatments on egg production, feed consumption, body weight, liver weight, visual fat score of the liver and liver composition are summarized in Table 5. Values shown are those obtained at the conclusion of the 4-week treatment period.

The hens fed the low-carbohydrate diets (Rations 3-6) had a significantly lower rate of egg production than those fed the control ration (Ration 1) or the high-protein, high-carbohydrate ration (Ration 2). The reduction in production rate was evident within 4 days from the start of the experiment. This reduction was less pronounced in the group fed the egg ration (Ration 3).

Feed consumption was affected by the rations used. Hens fed the cooked egg ration consumed almost twice as much feed as the birds fed the control ration thus having essentially the same caloric intake. The hens fed the ration containing skim milk powder consumed approximately three-quarters as much feed as the controls while those fed dried whey and vitamin-free casein (Ration 5) or animal products with dried whey and vitamin-free casein

Table 5. Effects of low carbohydrate diets on egg production, feed consumption, body weight, liver weight, visual fat score of the liver and liver composition of laying hens

	RATION NUMBER					
	1	2	3	4	5	6
Egg production, %	67[b1]	74[a]	24[c]	16[c]	14[c]	17[c]
Feed consumption, kg	44.4[b]	46.8[b]	87.0[a]	34.2[b]	18.5[b]	18.7[b]
Initial body wt , g	1871	1892	2088	1954	2019	1962
Final body wt , g	1950[ab]	2090[a]	2040[a]	1714[bc]	1621[c]	1487[c]
Abdominal fat pad wt , g	114.9[b]	114.9[b]	130.7[a]	66.8[c]	71.6[d]	76.7[e]
Ovary and oviduct wt , g	114.1[a]	119.2[a]	47.4[b]	42.1[b]	9.6[c]	12.5[c]
LIVER DATA						
Fresh wt , g	60.0[a]	62.0[a]	56.7[a]	45.8[b]	38.6[b]	35.3[b]
g/100 g/body wt	3.07	2.99	2.76	2.67	2.44	2.41
Visual fat score	3.1[a]	2.5[b]	3.2[a]	2.0[bc]	1.6[cd]	1.3[d]
Dry matter, %	31.8[a]	29.1[ab]	32.3[a]	28.8[ab]	27.2[b]	26.9[b]
Protein, %	50.8[a]	60.8[b]	57.8[ab]	68.8[c]	73.3[c]	74.7[c]
Fat, %	36.5[a]	29.4[a]	30.0[a]	18.6[b]	13.1[b]	12.0[b]

[1]Row values with same superscript or no superscript are not significantly different by Duncan's Range Test ($P<0.05$).

(Ration 6) consumed less than half as much feed as the controls. Because of the reduced feed intake, caloric consumption on these "low-carbohydrate" rations was greatly restricted. No explanation for failure of the birds to consume enough to meet their energy requirements is evident; however, the birds fed these low-carbohydrate rations developed profuse diarrhea. This may have had an effect on feed consumption.

The treatments used had variable effects on changes in body weight during the experiment. The hens fed the control ration and the high-protein ration increased slightly in body weight and the group fed the egg ration maintained relatively stable body weight. Hens fed the other low-carbohydrate rations (Rations 4-6) showed significant losses in body weight. The magnitude of weight loss was closely associated with the level of feed intake.

The weight of the abdominal fat pads appeared to be closely related to changes that occurred in body weight. Hens fed the control ration, the high-protein ration and the egg ration had large fat pads while those on the other low-carbohydrate rations had significantly smaller abdominal fat pads.

The combined weights of the ovary and oviduct appeared to be directly related to the levels of production of each treatment and accounted for part of the weight reduction that occurred in the groups fed low-carbohydrate rations. The loss was greatest in the groups showing the largest

body weight loss (Rations 5 and 6).

Fresh liver weights were reduced in hens fed the low-carbohydrate rations (Rations 4, 5 and 6) but liver weights of birds fed the egg ration were not reduced. When calculated in relation to body size, there were no significant differences in liver size between treatments.

Visual fat scores of the livers indicated that the level of liver fat was reduced in three of the low-carbohydrate treatments (Ration 4, 5 and 6) as compared to the controls. The scores on the livers of the hens fed the egg ration were similar to those of the controls. No evidence of haematomas in either the controls or treatment groups was observed.

The composition of the livers was affected by the treatments used. Groups fed Rations 4, 5 and 6 contained a higher level of protein and a lower level of fat than was observed in the other treatments. It is interesting to note that the hens fed the egg ration (Ration 3) had liver fat levels similar to those fed the control ration.

Summary

Shaver Starcross 288 hens, 73 weeks of age, were fed rations of very low carbohydrate content to determine the influence that such treatments would have on production rate, body weight, liver composition and body fat reserves. The experiment was terminated after 28 days on treatment. The results obtained indicated:

1. Feeding rations of low-carbohydrate content caused a reduction in rate of egg production.
2. Of the groups fed low-carbohydrate rations, only those on the egg ration consumed enough feed to meet their energy needs. Birds fed the other low-carbohydrate rations reduced their daily caloric intake. This resulted in marked losses in body weight and reduced size of the abdominal fat pads as compared to the other treatments.
3. Fresh liver weights were significantly reduced when low-carbohydrate rations were fed. When liver weights were expressed on the basis of body weight, no significant differences were observed.
4. The level of fat in the livers of hens fed the egg ration was similar to that in the hens given the control and high-protein rations. Liver fat levels in the hens fed the other low-carbohydrate rations was markedly reduced. Visual fat scores of the livers were closely correlated with their actual fat content.

SECTION III

The effect of restricted feed intake on the composition of livers of laying hens.

Status of the problem

The previous experiment (Section II) failed to delineate the reason for the reduced lipid content of the livers of hens fed rations of low carbohydrate content. It appeared that the reductions that occurred may have been related to level of feed intake rather than the carbohydrate content of the ration. It therefore seemed desirable to determine the influence that caloric restriction might have on the amount of fat present in the liver. Consequently, an experiment was conducted to determine the effect of various levels of feed restriction on the fat content of the liver of mature laying hens that had completed their first laying year.

Experimental

Sixty four Shaver Starcross 288 laying hens, 80 weeks of age, that had finished their first year of production in laying batteries were randomly selected, leg-banded, weighed and divided into eight comparable groups. The birds were placed in floor pens 1.42 meters x 4.27 meters in size and a period of 5 days was allowed before beginning the experiment to permit the birds to become accustomed to the change from batteries to floor pens.

At the end of the 5-day acclimatization period, two groups were placed on each of the four treatments. The

treatments used involved feeding the hens in each group either 60, 70, 80 or 90 grams of laying mash (Table 1, Ration 1) per bird per day. One-half of the daily allotment was given at 7:30 a.m. and the rest at 1 p.m. each day. Because of an error that occurred in feeding, actual feed consumption values in three of the groups was slightly higher than specified. The actual amount of feed consumed is given in Table 6. Twice-daily feeding was used to ensure that all hens had an equal opportunity to consume their share of the daily feed allotment and to try to reduce the influence of "peck order" on feed consumption. The experiment was terminated after 4 weeks on treatment.

Eggs were collected four times daily so that hens on the more restricted feeding regimes would not have an opportunity to eat the eggs that had been laid and so that floor eggs would not become buried in the litter. A daily record was kept on egg production.

Throughout the experiment water was supplied ad libitum. Artificial lighting was provided for 14 hours per day but natural light also entered through the windows of the building. This extended average day length because the trial was conducted in July.

All hens were weighed after 2 weeks on treatment and at the conclusion of the experiment. A record was kept of egg production and mortality during the experiment.

At the conclusion of the experiment six hens from each replicate were sacrificed by cervical dislocation and their

livers were examined and visually scored. The livers were stored and analyzed in the same manner as was used in Section I.

Duncan's Multiple Range Test was applied to the liver composition data at the 0.05 level of probability as an evaluation of the analyses of variance and significance of differences. Details are shown in Appendix V.

Results and Discussion

The effect of feed restriction on egg production, body weight, liver weight and liver composition is summarized in Table 6. Since there were unequal numbers in the replicates due to mortality during the acclimatization period the body weight, liver weights and liver composition data includes only the values from six birds in each replicate.

No significant differences in rate of egg production were caused by two different levels of feed restriction imposed. This may reflect the fact that the body weight of the hens was high and egg production had already declined to a low level when the experiment commenced.

Restriction of feed intake had no effect on final body weight but resulted in a significant increase in loss of body weight. The fact that the hens in all treatments, including those being fed as much as they would consume (Treatment 1), lost weight during the 4-week experimental period may have been responsible for the failure to observe a difference in final body weight. It is also possible that the hens were not fully acclimatized to feeding and management

Table 6. Effect of feed restriction on egg production, body weight, wet liver weight and liver composition of laying hens.

	Treatment Number			
	1	2	3	4
Feed intake (g/bird/day)	85.0	83.6	71.2	62.6
Egg production (% HDP)	29.5	41.0	40.2	26.7
Initial body weight, g	1764	1823	1882	1859
Final body weight, g	1632	1705	1573	1512
Weight loss, g	132[a]	118[a]	309[b]	347[b]
LIVER				
Fresh weight, g	40.9[a1]	35.3[b]	30.8[c]	29.6[c]
g/100 g/body weight	2.56[a]	2.09[b]	1.99[b]	1.96[b]
Visual fat score	3.8	3.2	2.8	2.2
Dry matter, %	27.90	26.57	26.45	27.32
Fat, %	19.08	16.96	14.86	15.01
Protein, %	68.80[a]	73.06[b]	74.36[b]	74.81[b]

[1]Row values with the same superscript or no superscript are not significantly different by Duncan's Range Test ($P<0.05$).

in the floor pens and as a result reduced feed intake.

Differences between treatments in fresh liver weights were observed, and when calculated as a percentage of total body weight, livers from the control group were significantly larger. This contrasted the previous experiment where birds on the most restricted caloric intake did not have significantly lower liver weights.

Appraisal of fat content by means of visual fat scores indicated that fat content decreased as level of feed intake decreased. Birds on the most restricted caloric intake (Treatment 4) had lower liver fat scores than the controls. The fat scores were slightly higher for the controls in this experiment than they were in the previous experiment. This may have been because older birds, producing at a lower rate, were used in this experiment. No haematomas were seen in any of the livers examined.

Although visual fat scores on the livers indicated that caloric restriction resulted in lower fat levels, chemical determinations failed to show a significant decrease in fat content in the restricted groups. The protein content of the livers of all of the restricted groups was increased above that noted on the control ration. No differences in dry matter content of the livers were observed. The failure of restriction of feed intake to affect liver fat levels does not help to explain the influence of low-carbohydrate rations on liver composition obtained in the previous experiment. Further work with larger groups of

hens and more severe feed restriction might help to clarify the contribution that reduced caloric intake may have had on differences which occurred in hens fed the low-carbohydrate diets (Section II).

Summary

Shaver Starcross 288 pullets, 80 weeks of age, at the end of their first year of egg production were supplied a commercial-type laying ration at levels of 60, 70, 80 and 90 grams per bird per day; the highest level was equivalent to unrestricted feed intake. The experiment was continued for 28 days. At the end of the trial, livers from each treatment were examined and their composition was determined. The results indicated:

1. Varying levels of feed intake had no effect on final body weight in this experiment; however, liver size was smaller from birds in the restricted treatments and body weight-loss during the trial was significantly greater in the more severely restricted groups.
2. Visual liver scores indicated that as feed intake levels were reduced the fat content of the livers declined.
3. The levels of feed restriction used showed no significant difference on the deposition of fat in the livers of laying hens.

GENERAL DISCUSSION

The lack of effect of varying levels of RSM in the ration fed on mortality, production rate and feed efficiency and the very low incidence of HLS in these experiments suggests that a level of 15 percent Tower RSM may be safely used in laying rations. This reenforces the general recommendation of Clandinin et al. (1976) that Tower RSM may be used at levels up to 10 percent of laying rations.

The observation that liver composition was not affected by level of Tower RSM agrees with the findings of Olomu (1974) and Serrano (1976) who reported that the inclusion of different levels of Span RSM did not cause any increase in size of the liver or any increase in fat deposition. The general lack of effect of various levels of RSM on fat content of the liver even when a high incidence of HLS was observed (Olomu, 1974; Serrano, 1976) suggests that the disorder associated with RSM inclusion in laying rations is probably different than the fatty liver syndrome described by Couch (1956).

The lack of effect of lipotropic factors in reducing liver fat content is in agreement with previous studies (Wolford and Murphy, 1972; Jensen et al., 1974; Serrano, 1976). Since liver fat levels were not affected by inclusion of RSM in this experiment, it can probably be assumed that no deficiency of lipotropic factors was present in the unsupplemented rations.

The use of low-carbohydrate rations as a means to reduce liver fat content gave variable results. Three of the rations fed gave reduced weight of abdominal fat pads and lower liver fat levels; however, hens fed the egg ration had abdominal fat pads and liver fat levels comparable to those fed the high-carbohydrate rations. The differences may be attributed to differences in energy intake. Hens fed the egg rations increased feed intake sufficiently to meet their energy needs whereas the birds fed the other low-carbohydrate diets greatly reduced caloric intake. This led to a significant reduction in weight of abdominal fat pads and fat content of the livers. The above observations suggest that feeding low-carbohydrate diets to chickens will not cause reduced body tissue stores of fat provided they consume enough to meet their daily caloric needs. This is at variance with the suggestion of Atkins (1972) that humans on low-carbohydrate diets can consume as much as they wish and will continue to lose weight. This work would suggest that it is necessary with chickens that caloric intake be reduced if weight loss is to be achieved on low-carbohydrate rations and lends support to the opinion of the American Medical Association Council of Foods and Nutrition (1974) that weight loss among humans consuming low-carbohydrate diets was due to reduced caloric intake.

The lack of effect of feed restriction in lowering liver lipid content and egg production contradicts the

findings of Couch (1974). The lack of decline in egg production among hens on restricted caloric intake could be attributed to the stage of egg production of the hens. The hens were almost out of production when the experiment started. Since hens in all treatments lost weight over the experimental period, liver lipid levels were probably altered at all levels of caloric intake so that a decrease in the most restricted groups was not significantly different than those being fed ad libitum.

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APPENDIX I

Procedure for analysis of livers

Hens were fasted overnight before being selected for visual appraisal and surgical removal of the liver samples. In order that blood present in haematomas would not unduly affect the liver analyses damaged tissue was removed from the livers. Livers were then stored at -20°C until they were analyzed.

At the time of analysis, the livers were freeze-dried to a constant weight and percentage dry matter was calculated. Constant weight was attained after approximately 72 hours in the freeze-drier.

The freeze-dried livers were individually ground in a micro analytical mill. For fat determination, samples were digested with 6N HCl in a Gold-Fisch reflux apparatus for an hour in 600 ml beakers. A ratio of 50 ml HCl per 0.80 gram of liver sample was used for the digestion. The digesta was filtered through a No. 42 Whatman filter paper 11.5 cm in diameter and the flasks were thoroughly rinsed with hot distilled water. The filter paper was washed free of acid with hot distilled water and then left to air dry. All samples were digested in duplicate. The small individual liver weights dictated that this technique be employed rather than the aliquot method used previously by Olomu (1974) and Serrano (1976) who pooled their samples. The residue left on the filter paper was extracted for 12 hours with petroleum ether (30-60°C. B.P.) and fat content was

calculated using the A.O.A.C. method (1965).

For protein determination duplicate samples (0.20-0.25 g) of each freeze-dried liver were weighed and transferred to 800 ml Kjeldahl flasks. Protein was determined by A.O.A.C. methods (1965).

APPENDIX II

Analyses of variance - source, degrees of freedom (df) and mean squares - lipotropic experiment

		Mean Squares										
Source[1]	df	Mortality (Transformed) %	HHP %	HDP %	Feed conversion kg feed/doz eggs	Egg weight g	Haugh units	Specific Gravity	Body wt (initial)	Body wt (final)	Body wt gain g	error[2]
L	1	37.55	63.56	10.07	0.965E-01	0.25	60.09	0.699E-07	225.0	116.1	664.4	R/LP
P	3	6.43	51.50	40.98	0.723E-01	5.96	30.40	0.620E-05	2237.9	4501.0	4328.1	R/LP
LP	3	4.46	17.44	9.19	0.161E-01	0.53	6.02	0.352E-05	419.8	3330.4	4278.8	R/LP
R/[3]LP	8	55.99	184.32	100.57	0.831E-01	0.58	7.04	0.215E-05	991.3	2954.1	2853.6	-

		Mean Squares						
		Liver						
Score[1]	df	Visual Score	Absolute Weight g	g/100 g body wt	Dry Matter %	Protein %	Fat %	Error
L	1	0.417E-01	274.5	0.486E-01	55.01	43.2	165.3	R/LP
P	3	2.944	137.9	0.103	43.04	159.6	179.4	R/LP
LP	3	0.486	163.4	0.417	40.51	221.1	383.0	R/LP
R/LP	8	1.667	50.5	0.115	11.49	52.4	82.0	C/R/LP
C/R/LP	80	0.421	175.6	0.240	23.01	114.6	157.2	-

[1]L-lipotropic levels; P-rapeseed meal levels; R-replicates; C-chickens.

[2]Denominator of mean square used in significance testing.

[3]/ signifies "within."

APPENDIX III

Analysis of covariance for percent fat adjusted for visual score - source, degrees of freedom (df) and mean squares - lipotropic experiment

Source[1]	df	Mean Squares Liver fat %
L	1	218.5
P	3	20.0
LP	3	223.3
Covariate[2]		4200.6
Residual[3]	87	102.8

[1]L-lipotropic level; P-rapeseed meal levels.

[2]Adjustment of liver fat for visual score.

[3]Chickens within lipotropic and rapeseed meal levels (C/LP).

APPENDIX IV

Analyses of variance - source, degrees of freedom (df) and mean squares - low-carbohydrate experiment

Source[1]	df	Mean Squares				
		Body wt (initial) g	Body wt (final) g	Abdominal fat pad wt g	Ovary & Oviduct wt g	error[2]
T	5	65046	0.600E+06	7850.7	23333	C/T
C/[3]T	54	49679	78773	1893.1	1003.4	-

Source[1]	df	Mean Squares						
		Liver						
		Fresh wt	g/100 g body wt	Visual fat score	Dry matter %	Protein %	Fat %	error[2]
T	5	1307.3	0.754	6.137	50.65	894.9	1020.4	C/T
C/T	54	142.9	0.331	0.361	15.42	69.4	91.8	-

[1]T-treatment; C-chicken.

[2]Denominator of mean square used in significance testing.

[3]/ signifies "within."

APPENDIX IV (cont'd)

Analyses of variance - source, degrees of freedom (df) and mean squares - low-carbohydrate experiment

		Mean Squares	
Source[1]	df	Feed Consumption kg	error[2]
T	5	291.38	TP
P	1	88.56	TP
TP	5	32.6	-

		Mean Squares	
Source[1]	df	Egg Production % HDP	error[2]
T	5	1511.6	TW
W	3	5450.9	TW
TW	15	767.4	D/TW
D/[3]TW	90	81.9	-

[1]T-treatment; P-time period; W-week; D-day.
[2]Denominator of mean square used in significance testing.
[3]/signifies "within."

APPENDIX V

Analyses of variance - source, degrees of freedom (df) and mean squares for traits measured in the feed restriction experiment

		Mean Squares			
Source[1]	df	Body wt (initial) g	Body wt (final) g	Weight loss g	error[2]
T	3	31858	81250	0.168E+06	P/T
P/[3]T	4	46731	24762	20427	C/PT
C/PT	40	90204	54814	27280	-

		Mean Squares	
Source[1]	df	Egg Production % HDP	error[2]
T	3	3121.82	P/T
P/T	4	1387.30	-
D	27	791.41	CP/T
DT	81	361.61	CP/T
CP/T	223	337.67	-

		Mean Squares (Liver)						
Source[1]	df	Absolute weight g	g/100 g body wt	Visual Score	Dry Matter %	Fat %	Protein %	error[2]
T	3	315.26	0.937	5.72	5.53	47.16	90.06	P/T
P/T	4	9.24	0.800E-01	1.46	4.53	12.21	12.44	C/PT
C/PT	40	30.58	0.118	0.72	5.70	34.56	36.98	-

[1]P-pens; T-treatment; C-chicken; D-day.

[2]Denominator of mean square used in significance testing.

[3]/ signifies "within."